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Valorization of organic carbon in primary sludge via semi-continuous dark fermentation: First step to establish a wastewater biorefinery

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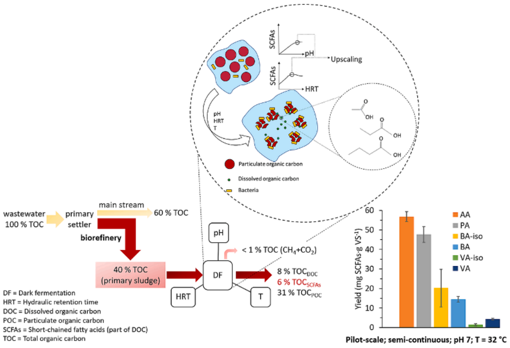
HIGHLIGHTS

- Higher accumulation of fatty acids at retention times of less than 48 h.
- Both pH and temperature had an effect on the selective production of fatty acids.
- Neutral to slightly alkaline pHs led to highest yields of individual fatty acids.
- Upscaling of dark fermentation showed a steady-state yield of 150 mg VFAs•gVS$^{-1.}$
- Significant improvement of acetic and propionic acid yields at 32 °C.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, lab-scale, bench-scale, and pilot-scale experiments were carried out to optimize short-chain fatty acids production from primary sludge. Batch tests showed the requirement of short retention times and semi-continuous operation mode showed a plateau of maximum daily productivity at 36-hours hydraulic retention time with minimal methanation. Optimization from pH 5 to pH 10 at 36 h-hydraulic retention time under long-term semi-continuous operating mode revealed that production of short-chain fatty acids was pH dependent and highest yields could be achieved at pH 7 by establishing optimum redox conditions for fermentation. Pilot-scale experiments at 32 °C showed that daily productivity (3.1 $g{\bullet}L^{-1}_{reactor}{\bullet}d^{-1}_{HRT}$) and yields (150 $mg{\bullet}g^{-1}_{VS}$; OLR = 21 $g_{VS}{\bullet}L^{-1}_{reactor}{\bullet}d^{-1}_{HRT}$; pH 7) of short-chain fatty acids could be significantly improved, specifically for acetic and propionic acids. From these results, a robust dark fermentation step for recovery of valuable products from the solids treatment step in a biorefinery can be achieved.

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1. Introduction

Lately, resource recovery from wastewater has gained increasing interest in research prompted by the depletion of non-renewable resources (Kehrein et al., 2020; Kleerebezem et al., 2015). Municipal wastewater treatment plants (WWTPs) have been traditionally developed to remove pollutant loads like carbon (C), nitrogen (N), and phosphorus (P) to prevent surface water pollution. However, the need for sustainable development has made municipal wastewater an untapped source for resource recovery (Parchami et al., 2020). Redesigning WWTPs to wastewater biorefineries (WWBrs) would realize such a necessity and directly address the environmental challenges associated with WWTPs by shifting from a linear to a circular economy (Sarkar et al., 2020).

To realize the idea of WWBrs, organic carbon needs to be valorized effectively as it can contribute directly to the production of value-added products. Organic carbon collected as sludge in a WWTP is typically converted to biogas (CH_4 and CO_2) during anaerobic digestion. In a conventional WWTP, around 30 % of influent chemical oxygen demand (COD) is converted to CO_2 in the biological treatment step, and approximately 33 % is converted to biogas (60 % CH_4 and 40 % CO_2) (Wan et al., 2016). Although biogas production via anaerobic digestion is a state-of-the-art process that provides a pathway for energy recovery, the metabolic intermediates during anaerobic fermentation (i.e., short-chain fatty acids (SCFAs)) have attracted increasing attention due to their higher value compared to biogas (Wainaina et al., 2019). SCFAs can be used as a carbon and/or energy source in many applications, for example, biological denitrification, production of biodiesel, current production in microbial fuel cells (MFCs), hydrogen production in microbial electrolysis cells (MECs), and synthesis of polyhydroxyalkanoates (PHAs) (Chen et al., 2012; Hackbarth et al., 2023; Lim et al., 2006; Sekoai et al., 2021). Dark fermentation has shown to be a highly effective biological approach due to its environmental friendliness and cost-effectiveness in producing SCFAs under anaerobic conditions. These SCFAs can serve as precursors to facilitate valuable products‘ recovery (Ramos-Suarez et al., 2021).

During fermentation, hydrolysis and acidogenesis occur in series with the former involving the breakdown of complex macromolecules like proteins, carbohydrates, and fats into simpler molecules, making them available for growth metabolism (Eastman & Ferguson, 1981). The considerable faster growth rate of acidogenic bacteria in comparison to methanogenic archaea provide a pathway towards SCFAs accumulation by controlling parameters like hydraulic retention time (HRT), pH, and temperature. For HRT, there are quite a few factors that need to be considered to enhance SCFAs production by acidogenic bacteria such as 1) providing adequate time for hydrolytic bacteria to breakdown particulate organic carbon (POC) and make it available to acidogenic bacteria for consumption; 2) preventing washout of acidogenic bacteria before SCFAs are produced or reach a plateau of maximum production; and 3) mitigating the growth of methanogens to not lose SCFA to methane production. In the case of pH, alkaline conditions can also serve as a pre-treatment similar to thermal, chemical, ultrasonic, and thermochemical pre-treatments to enhance the faster breakdown of POC and promote dark fermentation (Li et al., 2014). In addition, pH has shown to be highly influential in establishing favorable conditions for microbial communities and instigating changes in metabolic pathways leading to different fractions of SCFAs (Feng et al., 2018; Feng et al., 2009). Also, changes in temperature can also cause significant shift in microflora and can selectively increase concentrations of SCFAs (Huang et al., 2021a).

Primary sludge (PS) generated via mechanical separation of wastewater after screening and grit chamber in a primary sedimentation tank is rich in organic content due to the presence of faeces, vegetables, fruits, textiles, paper, etc. (Yuan et al., 2006). Around 70 % of the sludge production in a WWTP arises from PS making its valorization an essential step in establishing a WWBr. Within the scope of this project, focus is directed towards establishing dark fermentation as a promising and stable process to treat PS in a semi-continuous operation. Since wastewater can undergo significant changes on a daily basis, the composition of PS will inevitably change. The impact of such variable conditions on dark fermentation has to be tested as it is a crucial step in the long-term functioning of a WWBr. Studies conducted on PS have shown that alkaline pH-values improve the solubilization of macromolecules and promote acidogenesis (Wu et al., 2009, 2010). For alkaline fermentation (pH 10), the need of longer HRTs have been reported (5 days) whereas, Wang et al., 2021 showed that at pH 9.5, high productivity could be achieved in 2 days. Other studies have shown that mildly acidic conditions with long HRTs can also provide favorable conditions for acidogenesis (Bouzas et al., 2002; Q. Yuan et al., 2010). Concerning temperature, in sludge fermentation, higher temperatures (from mesophilic to thermophilic) can enhance yields of SCFAs (Huang et al., 2021b) but a contrary finding was observed by Crutchik et al., 2018, wherein the yields of SCFAs declined above the mesophilic range. Such discrepancies in key parameters need to be addressed by careful optimization.

Integration of crucial parameters such as pH, HRT, temperature, and operational stability over long-term changing PS conditions in a real WWTP and their effect on SCFAs productivity and yields have not been reported in literature so far. However, knowledge of the corresponding process stability is of great importance for assessing the possible recovery of commercial products (like H_2 and PHA) from SCFAs. In this study, experiments were done systematically from lab-scale to bench-scale to pilot-scale to achieve maximum daily productivities and yields of total and individual SCFAs. Batch-scale experiments were performed to observe changes in SCFAs during long HRTs. Bench-scale experiments were carried out to optimize HRT and pH while also comparing PS from two different WWTPs. The pilot-scale process ran under optimized conditions and was tested for the influence of temperature.

2. Materials and methods

2.1. Experimental setup

In this study, experiments were performed using three different reactor setups and distinguished into different operation phases (OP). The information on the different OPs is listed in Table 1. The initial optimization experiments (OP I) were carried out using six stirred lab-scale batch reactors with a working volume (V_w) of 1.8 L each (V_{tot} = 2 L), the description of which can be found elsewhere (Ali et al., 2021). Further experiments in semi-continuous mode were performed using a bench-scale reactor with a total volume (V_{tot}) of 40 L (OPs II, III, IV). V_w was set at 35 L for phase II, and 30 L for OPs III and IV. The reduced V_w was to cope with the shortage of PS during OPs III and IV. A screw pump (Netzsch, Germany) with a flow rate (Q_{pump}) of 400 $L{\bullet}h^{-1}$ (11 to 13 recirculations per hour) was used for the homogenization of the PS as well as to harvest and feed the primary sludge at regular intervals to maintain specific HRTs. The optimized results from the lab-scale and bench-scale setups were then transferred to a stainless-steel pilot-scale reactor insulated by polyurethane with V_{tot} of 300 L and V_w of 200 L (OP V). For the pilot-scale reactor, V_w was fixed based on the load of the PS generated under a constant influent flow rate of wastewater into the WWBr (1 $m^3{\bullet}h^{-1}$). Fig. 1 shows a schematic representation of the pilot-scale reactor setup.

For the pilot-scale setup, Q_{pump} of the screw pump was set to 2300 $L{\bullet}h^{-1}$ (12 recirculations per hour) and 4300 $L{\bullet}h^{-1}$ (22 recirculations per hour) for phases V-A and V-B, respectively. At pilot-scale, a primary sedimentation tank was directly coupled with the dark fermentation reactor. Primary sludge generated in the primary sedimentation tank was fed using the screw pump and the same was used to harvest the hydrolyzate to maintain specific HRTs (see Fig. 1). Two dosage pumps, P1 and P2 (Prominent, Germany), were implemented in the recirculation line of the setup to control the pH-value. The pH was adjusted using 5 M HCl and NaOH solutions. A pH meter (Greisinger, Germany) was

Table 1

. Conditions for different OPs performed in batch (I-A to I-C) and semi-continuous modes (II-A to V-B)) (*temperature was controlled). V_R: reactor volume; HRT: hydraulic retention time; OP: Operation phase.

OP	V_R (L)	Mode	HRT (h)	pH	T (°C)	Sludge
I-A	2	Batch	168	5.40 ± 0.11	30.0	Buesnau
I-B	2	Batch	168	5.81 ± 0.18	30.0	Buesnau
I-C	2	Batch	168	6.77 ± 0.30	30.0	Buesnau
II-A	40	Semi-continuous	12	5.94 ± 0.01	23.2 ± 1.4	Buesnau
II-B	40	Semi-continuous	18	5.94 ± 0.01	23.2 ± 1.4	Buesnau
II-C	40	Semi-continuous	24	5.96 ± 0.01	24.1 ± 0.9	Buesnau
II-D	40	Semi-continuous	36	5.96 ± 0.01	24.1 ± 0.9	Buesnau
II-E	40	Semi-continuous	48	5.96 ± 0.01	24.1 ± 0.9	Buesnau
III-A	40	Semi-continuous	36	5.21 ± 0.01	22.2 ± 0.8*	Buesnau
III-B	40	Semi-continuous	36	6.11 ± 0.27	23.6 ± 1.6*	Buesnau
III-C	40	Semi-continuous	36	7.11 ± 0.26	24.6 ± 1.4*	Buesnau
III-D	40	Semi-continuous	36	8.13 ± 0.13	24.8 ± 0.4*	Buesnau
III-E	40	Semi-continuous	36	8.97 ± 0.06	22.6 ± 1.5*	Buesnau
III-F	40	Semi-continuous	36	9.91 ± 0.09	24.0 ± 0.6*	Buesnau
IV-A	40	Semi-continuous	36	5.87 ± 0.10	23.1 ± 0.8*	Sindelfingen
IV-B	40	Semi-continuous	36	8.01 ± 0.15	23.1 ± 0.8*	Sindelfingen
IV-C	40	Semi-continuous	36	9.98 ± 0.22	23.1 ± 0.8*	Sindelfingen
V-A	300	Semi-continuous	36	7.11 ± 0.15	25.2 ± 1.9	Buesnau
V-B	300	Semi-continuous	36	6.89 ± 0.01	32.4 ± 0.4	Buesnau

also installed in the recirculation line, and the data was monitored using a data acquisition software (Volker Preyl, Germany). The flow rate of the gas produced was recorded using a drum-type gas meter (Ritter, Germany). Gas samples were collected regularly from the headspace of the reactor, and the composition of the gases was analyzed using a gas chromatograph coupled with a helium ionization detector (HP 6890 series, USA). The temperature in the reactor was controlled using a thermostat (Julabo GmbH, Germany) connected to a coil within the reactor vessel (for the pilot-scale, temperature control was carried out by varying the flow rate of the pump (see section 3.4)).

2.2. Analytical methods

Due to temporary availability constraints during the study, two sources of PS were used for the experiments. For phases I, II, III, and V, the PS was obtained from Buesnau WWTP (Germany), meanwhile for phase IV, the PS was taken from Sindelfingen WWTP (Germany). Parameters in the sludge and hydrolyzate were classified into total and dissolved parameters, determined from raw or filtered samples, respectively. To measure the dissolved parameters, samples were first centrifuged at 4000 rpm. Then, the supernatant was filtered off using a glass fiber membrane with a pore size of 1 μm, followed by a regenerated cellulose (RC) membrane with a pore size of 0.45 μm. Total organic carbon (TOC), total Kjeldahl Nitrogen (TKN) and total phosphorus (TP) were measured according to DIN EN 13137, DIN EN 16169, and DIN EN ISO 17294–2, respectively. Total solids (TS) and volatile solids (VS) were measured using standard methods (DIN 38414). Dissolved parameters such as dissolved organic nitrogen (DOC) and ammonium nitrogen (NH_4^+-N) were measured in accordance with DIN EN 1484 and DIN 38406–5-2, respectively. All parameters mentioned above have been measured twice. For batch tests, duplicate experiments were carried out and for semi-continuous experiments, two to five values were used for reproducibility (see section 2.3).

In addition, lactic acid (LA) and SCFAs from the filtered samples were detected using ion chromatography (IC) systems 881 Compact Pro (Metrohm, Switzerland). LA had a high instability in production and was found only in very low concentrations, less than 80 mg•L^{-1}. For this reason, LA was not included in any calculations defined by equations (ii), (iii) and (iv) in subsection 2.4. Methanol and ethanol were occasionally measured using a gas chromatograph coupled with a flame ionization detector (Agilent 7890B, USA) and concentrations were always less than the detection limit of 100 mg/L. A portable multimeter (WTW Multi 350i, Xylem, USA) was used to corroborate the pH-value. Dissolved oxygen (Greisinger, Germany) and oxidation reduction

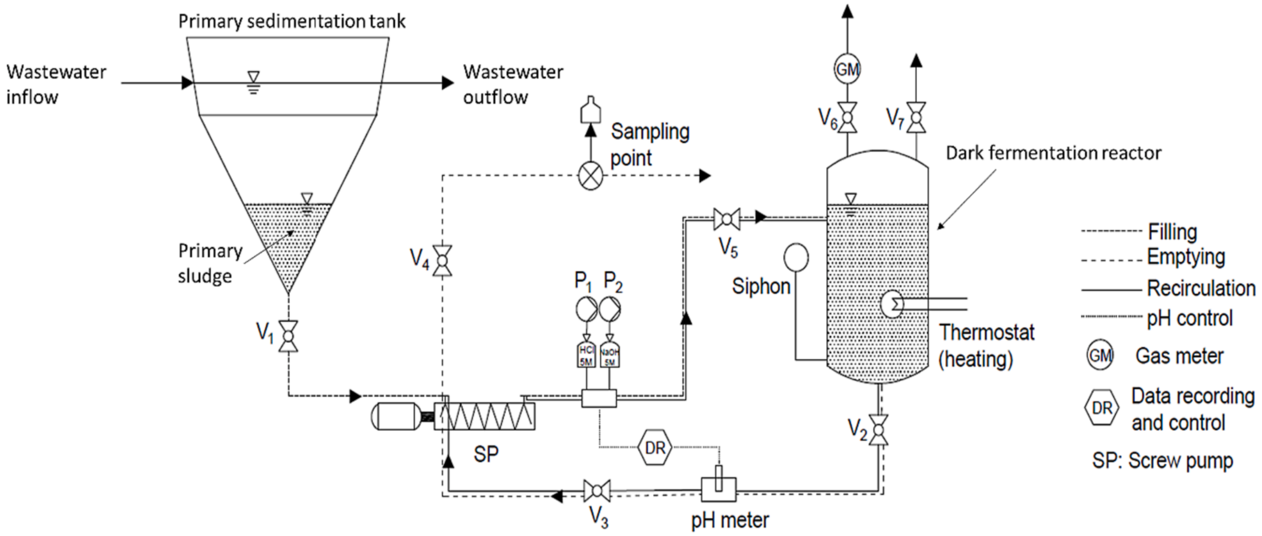

Fig. 1. . Schematic representation of pilot-scale dark fermentation reactor coupled with primary sedimentation tank.

potential (WTW, Multi 350i, Xylem, USA) were measured occasionally in the semi-continuous operation mode to ensure anaerobic conditions since the reactor underwent regular feeding. DO was approximately 0 $mg\bullet L^{-1}$ and ORP was between minus 350 mV and minus 390 mV.

2.3. Experimental conditions

The batch experiments were performed in duplicates to test reproducibility. For the semi-continuous operation mode, a certain steady-state phase was established for the production of SCFAs to assure process stability. To establish reproducibility of each phase, an OP was carried out for a period of at least two times the chosen HRT. The steady-state (arbitrary steady state, SS_{ar}) itself was established arbitrarily and defined as the spot where the coefficient of variation (CV) for total short-chain fatty acids (tSCFAs) productivity was equal to or below 10 %. It is to be noted that when calculating yield values for individual short-chain fatty acids (SCFAs), the values were chosen when SS_{ar} was met for tSCFAs. However, there was one instance for phase IV-A where SS_{ar} was not completely met ($SS_{ar} = 16$ %). The combination of the two conditions, HRT and CV, are represented in the following equation,

$$SS_{ar} = \{OP \geq HRT\,(h) \times 2 \nabla CV(d[tSCFAs] \leq 10\,\%\} \tag{i}$$

The OPs performed in this study are listed in Table 1. OPs I and II were done to optimize the HRT. OPs III and IV were performed to optimize pH. The temperature was not controlled for OP I as the experiments were performed during warm weather conditions. For OP II and III, the temperature was controlled due to cold weather conditions in order to provide comparability. The OP V-A was conducted under optimum HRT and pH, while the OP V-B was carried out to test the effect of temperature.

2.4. Data interpretation

The study mainly focuses on daily productivity, and the yields of SCFAs, CH_4, and H_2.

2.4.1. Daily productivity

For batch experiments, the apparent daily productivity, P_X, was calculated using the following equation,

$$P_X\left[mg \bullet L^{-1} \bullet d^{-1}\right] = r_X\left[mg \bullet L^{-1} \bullet d^{-1}\right] \times \frac{V_W\,[L]}{V_{f/h}\,[L]} = \frac{c_{X,t_i}\left[mg \bullet L^{-1}\right] - c_{X,t_o}\left[mg \bullet L^{-1}\right]}{t_i\,[d] - t_o[d]} \tag{ii}$$

i (d) = 1,2,3…nwhere, r_x is the reaction rate, the subscript X refers to the dissolved parameters whose daily productivity was calculated, V_W is the working volume and $V_{f/H}$ is the volume fed or harvested (for batch systems, there is no feeding, hence $V_W = V_{f/H}$), c_{X,t_i} and c_{X,t_0} represent the mass concentrations of SCFAs or tSCFAs at the time (t_i) and initial time (t_0). tSCFAs is the sum of mass concentrations of individual SCFAs such as acetic acid (AA), propionic acid (PA), *iso*-butyric acid (BA-iso), butyric acid (BA), *iso*-valeric acid (VA-iso), and valeric acid (VA).

For semi-continuous operation, the daily productivity, P_X, was estimated using the following equation,

$$P_X\left[mg \bullet L^{-1} \bullet d^{-1}\right] = r_X\left[mg \bullet L^{-1} \bullet d^{-1}\right] \times \frac{V_W\,[L]}{V_{f/h}\,[L]} = \frac{c_{X,out}\left[mg \bullet L^{-1}\right] - c_{X,in}\left[mg \bullet L^{-1}\right]}{dt\,[d]} \tag{iii}$$

where $c_{X,out}$ and $c_{X,in}$ represent the mass concentrations of SCFAs or tSCFAs in the effluent and influent of the reactor, respectively, and dt is the duration of each cycle, which is approximately one day.

2.4.2. Yield

The yield, $Y_{X/VS,}$ was calculated using the following equation,

$$Y_{X/VS}\left[mg \bullet g^{-1}\right] = \frac{r_X\left[mg \bullet L^{-1} \bullet d^{-1}\right]}{OLR\left[g_{vs} \bullet L^{-1} \bullet d^{-1}\right]} \tag{iv}$$

where OLR is the organic loading rate, expressed as g of volatile solids fed per liter and day.

The average gas yield for CH_4 and H_2 was estimated using the following equations,

$$Y_{CH_4,\,H_2,avg/VS}\left[NmL \bullet g^{-1}\right] = \frac{Q_{CH_4,H_2}\left[NmL \bullet d^{-1}\right]}{OLR\left[g_{vs} \bullet L^{-1} \bullet d^{-1}\right] \times V_W[L]} \tag{v}$$

To estimate the acidification fraction, f_{DOC} was used to show the overall contribution of SCFAs to DOC in the hydrolyzate, expressed as a ratio between SCFAs as carbon equivalents to DOC.

$$f_{DOC}[\%] = \frac{C_{SCFAs,out}\left[mg \bullet L^{-1}\right]}{DOC,out\left[mg \bullet L^{-1}\right]} \times 100 \tag{vii}$$

where $C_{SCFAs,out}$ and $C_{DOC,out}$ represent the mass concentrations of SCFAs as carbon equivalents and DOC in the outflow, respectively. The results for f_{DOC} can be found in the E-supplementary data.

3. Results and discussions

3.1. Characteristics of primary sludge and process stability during semi-continuous operation

In general, PS underwent notable changes as experiments were performed over longer periods of time and at different times during the year. As experiments were performed over a long-time span, Table 2 shows the feeding characteristics of PS which are essentially the influent concentrations for different OPs. For experiments performed with PS obtained from Buesnau, sludge was taken from the primary sedimentation tank on a daily basis. On the other hand, for experiments performed with PS sourced from Sindelfingen, the PS was stored in a storage tank. The latter PS underwent changes during storage, and the parameters in the influent were measured every day to obtain the actual values of P_X and $Y_{X/VS}$ (see E-supplementary data for the parameters in the influent for different OPs).

Since terminal treatment steps, say hydrogen and bioplastic production, require stable production of tSCFAs and, more importantly individual SCFAs, it was essential to evaluate process stability to observe if SCFAs underwent significant changes in coefficient of variation (CV). In all OPs at semi-continuous feeding mode, the dominant SCFAs produced were AA, PA, BA, and BA-iso, with VA and VA-iso having mass fractions less than 10 %. In most cases during the SS_{ar}, CV for individual SCFAs (dominant fractions) was less than 15 %, with some exceptions. Maintaining ideal conditions by appropriately controlling parameters like HRT, pH and temperature can ensure long-term functioning of dark fermentation with low CVs.

3.2. Evaluation of hydraulic retention time at batch and semi-continuous operation modes

OP-I was performed in a batch mode to observe the trend of SCFAs and methane productivity (see Fig. 2) in dependency on the pH-value (5.4 ± 0.1 (OP I-A), 5.8 ± 0.2 (OP I-B), and 6.8 ± 0.3 (OP I-C)). Production of C2 to C5 fatty acids was observed in all cases with branched SCFA, BA-iso being more predominant at I-C at 18 h. The SCFAs were generally produced from the solubilization of macromolecules, proteins, carbohydrates, and lipids, which were then quickly fermented to pyruvate via glycolysis and then to SCFAs (Fang et al., 2020; Yu et al., 2003). The fraction of AA:PA:BA was close to 90 % for I-A and I-B. This fraction

Table 2

. Characteristics of the PS at different OPs. σ denotes standard deviations determined throughout the respective OPs. PS: Primary sludge; OP: Operation phase; TS: Total solids; VS: Volatile solids; TOC: Total organic carbon; TKN: Total Kjeldahl nitrogen; TP: Total phosphoru; OLR: Organic loading rate.

OP	g•L^{-1}			mg•L^{-1}		C:N	OLR	OLR
	TS	VS	TOC	TKN	TP	(g_{TOC}•g^{1}_{TKN-})	(g_{VS}•L^{-1}•d^{-1})	(g_{TOC}•L^{-1}•d^{-1})
I-A, B, C	37 ± 2	33 ± 2	19 ± 1	–	–	–	–	–
II-A	29 ± 6	26 ± 6	13 ± 3	1012 ± 245	248 ± 64	13 ± 1	52 ± 12	26 ± 5
II-B	40 ± 4	34 ± 3	16 ± 1	1072 ± 225	348 ± 37	16 ± 5	45 ± 3	22 ± 2
II-C	35 ± 3	31 ± 2	15 ± 1	1286 ± 70	288 ± 14	12 ± 1	31 ± 2	15 ± 1
II-D							21 ± 1	10 ± 1
II-E							16 ± 1	8 ± 1
III-A	37	37	18	991 ± 133	27 ± 2	18 ± 3	25	12
III-B	37 ± 6	35 ± 5	17 ± 2	985 ± 155	43 ± 7	17	24 ± 3	11 ± 2
III-C	22 ± 6	20 ± 5	10 ± 3	642 ± 146	158 ± 31	16 ± 1	14 ± 4	7 ± 2
III-D, E, F	32 ± 7	27 ± 6	13 ± 3	775 ± 115	160 ± 72	17 ± 2	18 ± 4	9 ± 2
IV-A	30 ± 4	25 ± 4	11 ± 1	1060 ± 154	453 ± 50	10	17 ± 2	7 ± 1
IV-B	35 ± 2	29 ± 2	13 ± 1	1246 ± 66	512 ± 18	10 ± 1	19 ± 1	8
IV-C	48 ± 6	40 ± 5	17 ± 2	1725 ± 198	661 ± 66	10 ± 1	27 ± 4	11 ± 1
V-A, B	38 ± 5	32 ± 4	16 ± 2	831 ± 114	167 ± 107	19 ± 1	21 ± 3	10 ± 1

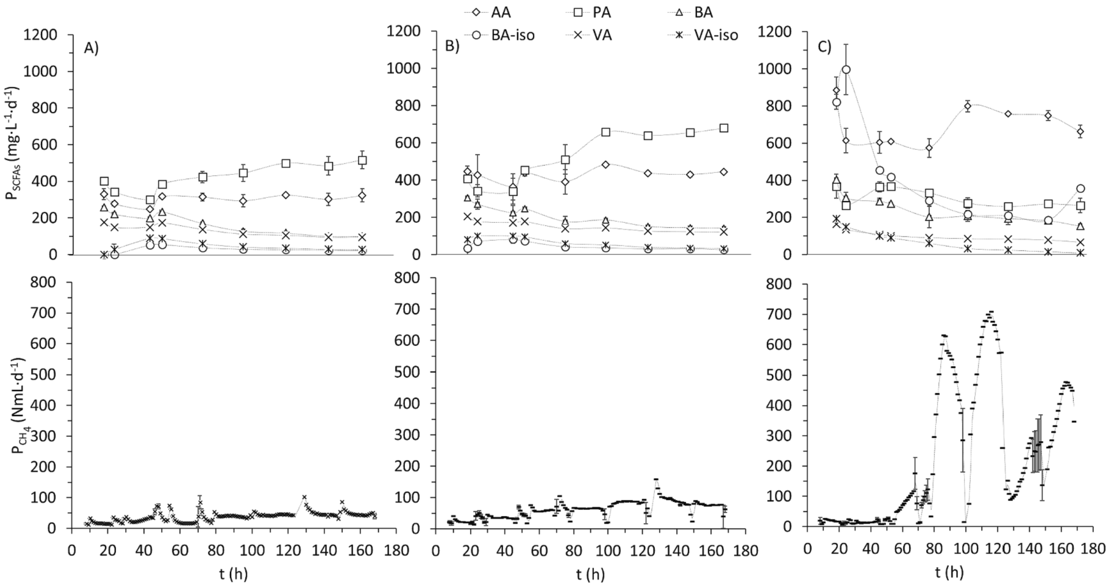

Fig. 2. . Daily productivities of individual short-chained fatty acids (SCFAs) (P_{SCFAs}) and CH_4 (P_{CH4}) for different pH-values, A) 5.4 ± 0.1 (operation phase (OP) I-A), B) 5.8 ± 0.2 (OP I-B), and C) 6.8 ± 0.3 (OP I-C) (refer Table 1 for detailed parameters).

was reduced to approximately 80 % in I-C due to higher production of BA-iso. After 24 h, the trend from I-A to I-C exhibited similar patterns except for PA and BA-iso in I-C (Fig. 2). PA was more dominant in I-A and I-B, while in I-C, AA was relatively higher. BA was the third most prevalent SCFA from I-A to I-C (see Fig. 2A, 2B and 2C). The dominance of BA-iso at pH above 6 was also shown in batch tests performed with sludge obtained from Bruchsal WWTP (Data for batch tests on Bruchsal PS can be found in E-supplementary data) and in some of the semi-continuous operation phases (OP III-C to III-E, IV-B, IV-C, and V). In this study, it could be deduced that accumulation of BA-iso appears to be prompted at pH-values above 6. The accumulation of BA-iso could be caused by its poor biodegradability compared to other SCFAs (Wu et al., 2010). However, BA-iso decreased significantly after 18 h implying its degradation (Fig. 2C). In a defined triculture anaerobic environment, Matthies and Schink (1992) observed isomerization of BA-iso to BA and the subsequent degradation of BA to AA and CH_4. These reactions were also noted to be an important first step in methanogenesis for the degradation of this branched SCFA. A study conducted by Chiu-Yue Lin, 1993 on sludge acclimatized with butyric and *iso*-butyric acid had also reported such a pathway for BA-iso degradation to AA. There appears to be a rise in P_{CH4} and P_{AA} at around 70 h after a significant decrease in $P_{BA\text{-}iso}$ at 40 h (Fig. 3C). However, the 30-h offset between degradation and production does not necessarily offer validation for such a pathway.

Higher P_{AA} in OP I-C could also be related with lower P_{CH_4} during the initial batch phase. For methanation, there are several pathways and among them, hydrogen and AA consumption have been known to be predominant in AD (Calise et al., 2020; Harirchi et al., 2022). Hydrogen ($4\ H_2 + CO_2$ -> $CH_4 + 2\ H_2O$) and AA (CH_3COOH–> $CH_4 + CO_2$) are substrates to methane production via hydrogenotrophic and acetoclastic methanogenesis, respectively. In OP I, P_{H_2} was relatively low and was between 3 and 10.5 NmL•d^{-1}. This would indicate the H_2 partial pressure was very low due to interspecies hydrogen transfer between obligatory producing and consuming bacteria (Boušková et al., 2005). The relatively lower productivities of AA in I-A and I-B would be a result of AA consumption during acetoclastic methanogenesis (Fig. 2A and 2B). However, with rising P_{CH_4} at 70 h in I-C, a decrease in P_{AA} should be expected but the contrary was observed (Fig. 3C). At this particular time (70 h), H_2 was absent from the headspace of the reactor and could be used as a substrate for both homoacetogens ($4H_2 + 2CO_2$ -> $2CH_3COOH$) and hydrogenotrophic methanogenesis (Karekar et al., 2022).

Nevertheless, daily productivity did not change notably for I-A and I-

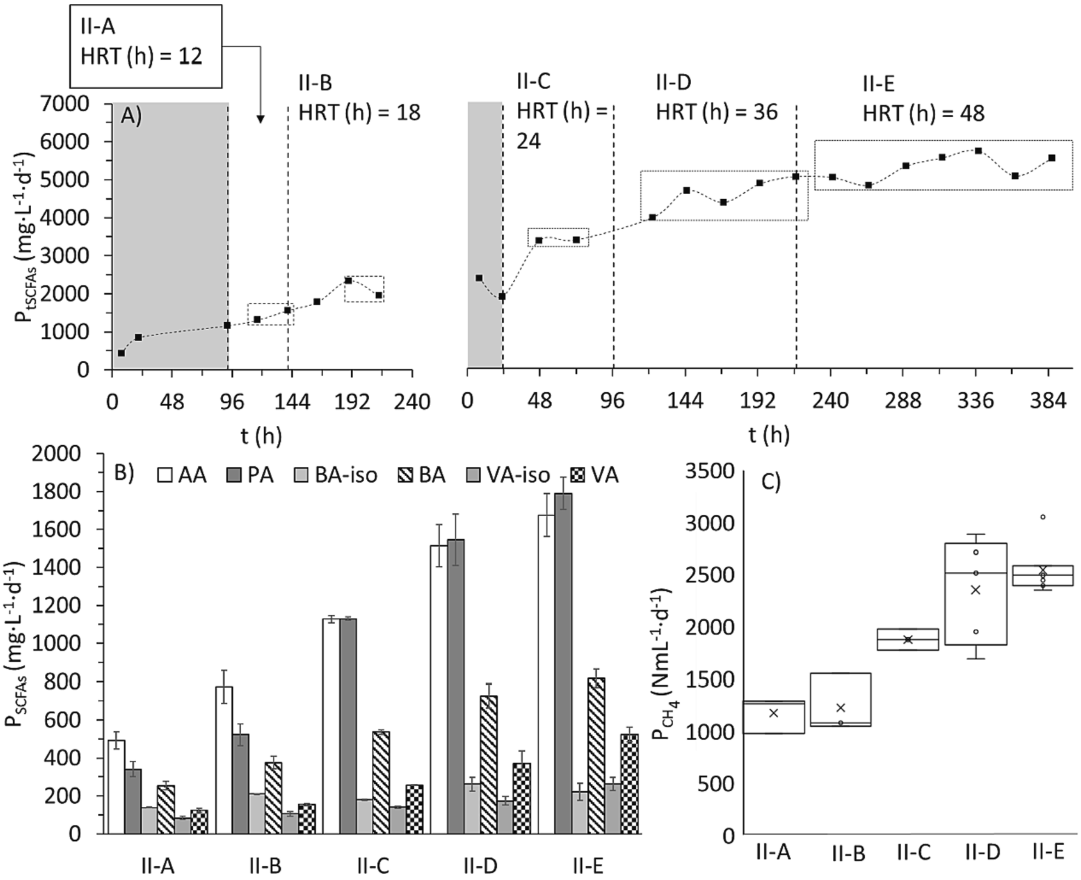

Fig. 3. . Daily productivities of A) total short-chained fatty acids (tSCFAs) (P_{tSCFAs}), B) individual SCFAs (P_{SCFAs}) and C) CH_4 (P_{CH4}) for hydraulic retention times from 12 h to 48 h at pH 6 (operation phase (OP)-II) (refer Table 1 for detailed parameters). The boxes indicate arbitrary steady-state and the shaded area shows the start-up batch phase.

B over the course of the batch. But there was an accumulation of SCFAs (specifically BA-iso and AA) in the first day of the batch at pH 6.8 ± 0.3 (I-C) (P_{tSCFAs} = 2901 ± 284 mg•L^{-1}•d^{-1}). Such a trend could also be observed with PS obtained from Bruchsal at pH 6 (P_{tSCFAs} = 995 ± 38 mg•L^{-1}•d^{-1} at 20 °C after 24 h) and pH 6.5 (P_{tSCFAs} = 1773 ± 202 mg•L^{-1}•d^{-1} at 20 °C after 24 h). For Bruchsal PS, there was an increase for C2 to C5 fatty acids after 24 h. At pH-values above 6, in both batch tests, higher SCFAs productivity along with higher methanation was observed. Although at pH-range 5 – 5.5, methanation is low, there is a drawback in terms of lower productivity. Therefore, pH 6 was chosen to maintain low methanation as well as relatively higher productivity for semi-continuous experiments to determine HRT in OP II.

Similar to the results from the batch operation phases, C2 to C5 fatty acids, linear, and branched SCFAs were produced with AA, PA, and BA having the highest concentrations from 12 h to 48 h and mass fractions (in sum) of more than 85 % in all cases. Fig. 3A and 3C show a linear increase in P_{tSCFAs} and P_{SCFAs} from 12 h to 36 h HRT (OP II-A to II-D) after which no significant difference was observed at 48 h. Interestingly, AA dominated until HRT of 18 h after which productivities of AA and PA were similar until 48 h HRT. This equivalence in productivity could be construed as a reduction of AA productivity which coincides with rising methanogenic activity, P_{CH_4} (see Fig. 3B and 3C) from HRT of 24 h. Similarly, Canziani et al., 1995 had reported a significant reduction of soluble COD (sCOD) concentrations at HRTs above 48 h due to increasing rates of methanation. At the steady state conditions SS_{ar}, P_{tSCFAs} reached maximum values of 4705 ± 384 mg•L^{-1}•d^{-1} and 5407 ± 305 mg•L^{-1}•d^{-1} at 36 h and 48 h HRT, respectively. In general, having low HRTs can be beneficial to extract the maximum productivity of SCFAs. Yehuda Miron et al., 1999 reported that to maintain acidogenesis, HRT less than 192 h was still appropriate. Furthermore, the minor increase in P_{tSCFAs} at 48 h compared to 36 h does not justify the higher OLR that could be processed at HRT 36 h. For this reason, HRT of 36 h was chosen to optimize pH.

3.3. *Effect of pH on short-chain fatty acids production and composition at semi-continuous operating mode*

For determination of the effect of pH on SCFAs production, the optimized HRT of 36 h (OP II-D) was chosen. In these experiments, pH values ranging from 5.2 to 10 were tested in OP III and IV. The results depicted in Fig. 4A show a notable increase of P_{tSCFAs} from pH 5.2 to pH 6.1 (III-A to III-B) by a factor of approx. 2.5, while the increase from 5.2 to neutral/slightly alkaline conditions of 7.1 (III-C) was even by a factor of 3.5 to 4, reaching the maximum productivity of almost 4 g• L^{-1}•d^{-1} even at low OLRs (OLR was different during the OPs and it was even lower at OP III-C, see Table 2). At moderately alkaline conditions with pH-values between 8.1 and 9 (OP III-D to III-E, and IV-B), a slightly lower P_{tSCFAs} was mostly maintained, showing no significant difference within this range except for variations among individual SCFAs. At pH 9.9/10 (OP III-F and IV-C), this productivity notably declined. Although pH-range 5 to 7 could be very well be optimum (based on batch and semi-continuous tests) at this HRT, it could be claimed that optimum HRT could be different at an alkaline pH-range. Wang et al., 2021 were able to achieve very high productivity at pH 9.5 within 48 h of fermentation for PS while Dahiya et al., 2015 could also observe high productivities at pH 10 within 10 h (until 48 h) of fermentation using food waste (inoculated with digested sludge). In this study, the decline at pH 9.9/10 (OP III-F and IV-C) shows the requirement of extended

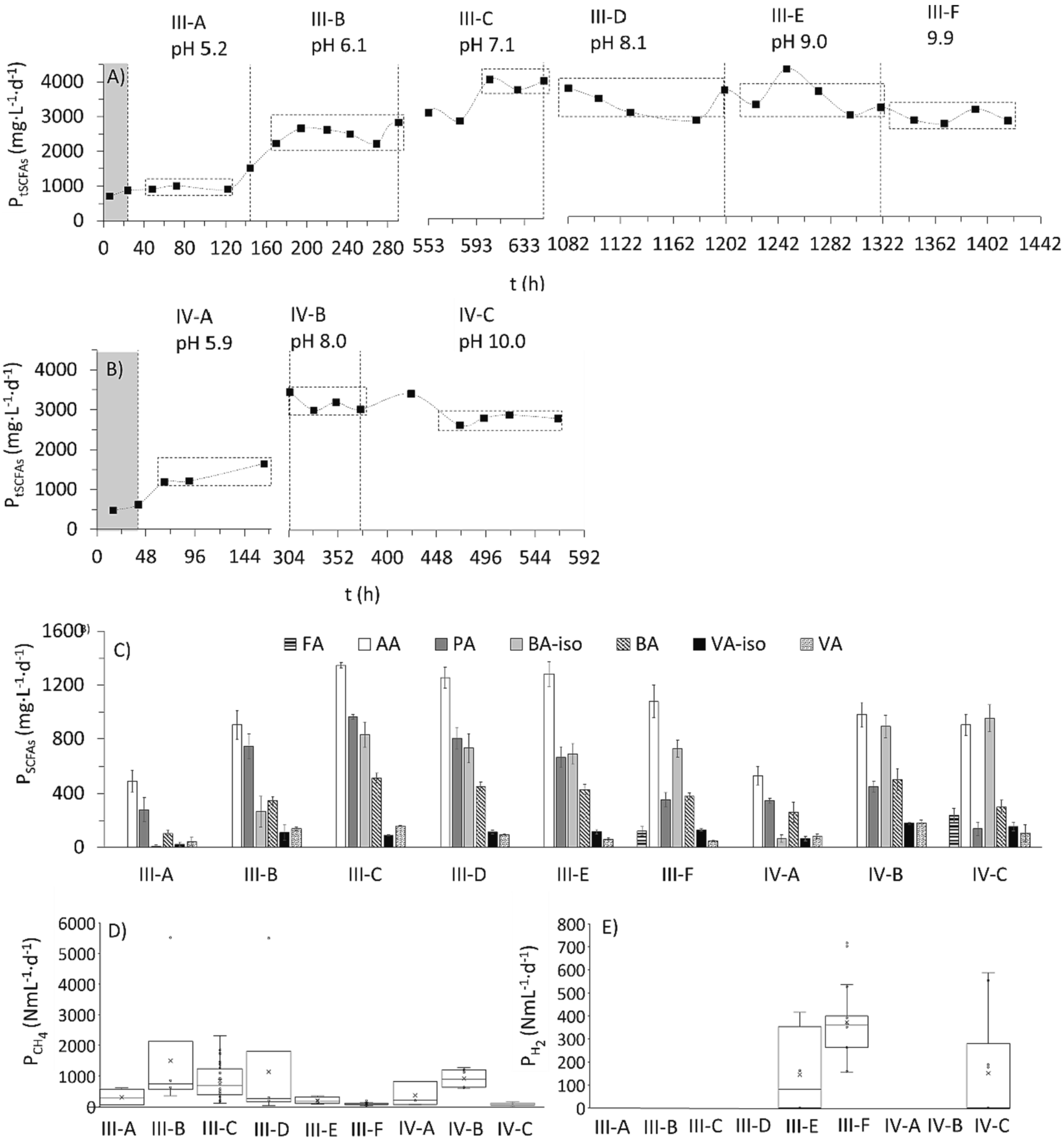

Fig. 4. . Daily productivities of A) total short-chained fatty acids (tSCFAs) (P_{tSCFAs}) for Buesnau primary sludge (PS) (operation phase (OP)-III), B) tSCFAs (P_{tSCFAs}) for Sindelfingen PS (OP-IV), C) individual SCFAs (P_{SCFAs}), D) CH_4 (P_{CH4}) and, E) H_2 (P_{H2}) for different pH-values (OP-III; pH 5.2 to 9.9 and OP-IV; pH 5.9 to 10.0) at hydraulic retention time of 36 h (refer Table 1 for detailed parameters). The boxes indicate arbitrary steady-state and the shaded area shows the start-up batch phase.

adaptation periods for acidogenesis. Wu et al., 2010 showed that at 5 days the highest yields of tSCFAs could be achieved at pH 10. The authors noted that, alkaline redox conditions favored faster breakdown of proteins to amino acids which were utilized by acidogenic bacteria. Similar results were also shown with excess sludge and waste activated sludge (Chen et al., 2006; Yuan et al., 2006). However, it is important to consider, that long retention times (between 5 and 8 days depending on the substrate) are required to achieve physicochemically aided biological effect. In this study, the decline at pH 9.9 (OP III-F)/pH 10 (OP IV-C) stated in Fig. 4A and 4B clearly arose mainly from the reduction in productivities of specifically PA and also from AA and BA. Such a trend was also observable from both kinds of PS as shown in OP-III and IV (compare OPs III and IV in Fig. 4B). Since several consortia of microorganisms are responsible for producing the individual SCFAs, the significant reduction in PA productivities could result from unfavorable conditions for the propionic bacterium, whose optimal range for growth could be between pH 6 and 8 (Horiuchi et al., 2002). Nevertheless, there was a general trend of higher P_{tSCFAs} at alkaline conditions compared to pH 5.2 and pH 6.1.

When considering the spectrum of SCFAs produced, mainly C2 to C5-acids were determined (Fig. 4B). However, the composition of SCFAs showed a notable change in the pH-range from pH 7.1 to 9.9 (OPs III-C to III-F) and pH-values of 8.1 and 10 (OPs IV-B and IV-C). AA, PA, BA-iso and BA production were significantly improved at pH 7.1 (Fig. 4C) above which the productivity declined. AA, PA, BA-iso, and BA are directly fermented from proteins, carbohydrates, and fats. However, VA and VA-iso are the results of protein degradation via reductive deamination or Stickland reaction (Chen et al., 2006). The higher productivities of these SCFAs at alkaline pHs compared to acidic ranges could be caused by higher solubilization of non-proteinaceous macromolecules (protein degradation appears to be low at OPs III and IV (see subsection 3.5)) at higher pHs as VA and VA-iso show no significant changes in productivity.

Inhibition of hydrogenotrophic methanogenesis prevented complete consumption of H_2 at alkaline conditions at OP III-E, III-F and IV-C (Fig. 4C and 4D). Cessation of methanogenesis at pH-values close to

10 also led to the accumulation of low concentrations of FA, which is another substrate for methanogenic archaea ($4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$) and could be seen in OPs III-F and IV-C (Fig. 4B). In this study, it is more or less clear that the major substrates for methane production are FA, H_2 and AA. Although alkaline pH-ranges (from pH 9 to pH 10) may hinder methanation, which should see a significant increase in SCFAs, there is a reduction or lack of increase in SCFAs production indicating that at short HRTs, alkaline pHs could hinder fermentation due to the requirement of extended adaptation periods and to see a considerable impact, HRT should be increased. However, providing optimum redox conditions for fermentation can significantly improve the productivity and yields. In conclusion, as neutral/slightly alkaline conditions showed the highest productivity of SCFAs in OP III-C with moderate methane production rates compared to acidic and alkaline conditions, pH in the neutral range was implemented for the pilot-scale setup.

3.4. *Upscaling to a 300 l-reactor under optimum conditions and effect of temperature*

During the upscaling experiment from 40 L to 300 L, temperature influence played a significant role due to the heat generated by the screw pump. The temperature was, thus, not controlled with an external thermostat, but rather the flow rate of the pump was raised to increase the temperature. Such an effect occurred because of energy loss due to frictional losses in the pump which was transformed into heat. This heat was prevented from dissipation by a polyurethane insulation of the reactor vessel leading to temperature containment in the reactor.

As expected, higher temperatures lead to increased reaction rates and higher production of SCFAs. It was also shown that temperature has a selective impact on SCFAs production. Depending on temperature requirements of the single microbial species of the consortium, biocoenosis composition will likely change with changing temperatures leading to an increase or decrease in production rates (Komemoto et al., 2009; Wainaina et al., 2019). In this study, raising the temperature by approximately 7 °C did not simply improve P_{tSCFAs} (Fig. 5A). While BA-iso, BA, VA-iso, and VA productivities were unaffected by the temperature increase, AA and PA productivities increased significantly by a factor of approx. 1.6 and 2.1, respectively (Fig. 5B). This probably shows that AA and PA-producing organisms were highly active around 32 °C. Research conducted on shifts in microflora in sludge at a genus level showed an increase in microbial communities that promoted production of individual SCFAs (including AA and PA) at 35 °C during sludge fermentation (Huang et al., 2021b). Experiments performed by Christensen et al., 2022 on PS showed a similar increase in the production of tSCFAs when the temperature rose from 30 °C to 35 °C. The authors showed an increase of 1.5 and 1.2 at pH-values of 5 and 7, respectively. In addition to acidogenesis, methanogenesis was also notably enhanced (Fig. 5C).

As explained above, the temperature increase was a result of a higher flow rate of the pump. Thus, with this increase in recirculation rate of the liquid, also the mixing intensity in the reactor was higher (see subsection 2.1). It is uncertain whether the increased recirculation rates did not positively affect reaction rates by affecting mass transfer in general or through sludge particle destruction due to increased shear stresses, or negatively by increasing mechanical damage of microorganisms through turbulence and shear forces. However, Ong et al., 2002 showed that there was no notable difference in SCFAs and biogas production between continuous and intermittent mixing at different impeller speeds. Similar experiments were also conducted to test the effect of recirculation duration/frequencies on biogas production from dairy manure with minimal effect of different recirculation frequencies (Rico et al., 2011). These findings, plus the fact that the observed overall increase in SCFAs production corresponds to the expected increase following the vańt Hoff-Arrhenius theory it is assumed, that the positive effect of temperature on production rates shown in this work are mainly attributed to temperature.

3.5. *Yields for bench-scale and pilot-scale setups at semi-continuous operating mode*

The yields calculated for all OPs are depicted in Fig. 6A. Maximum yields achieved for HRT optimization (OP -II) were at 36 h and 48 h as shown in Fig. 6A (36 h was chosen for reasons mentioned in subsection 3.2). At bench-scale (OP III-C), the highest yield of around 200 $mg_{SCFAs} \bullet g_{VS}^{-1}$ was achieved at pH 7.1. In the upscaled reactor system under optimum conditions (HRT of 36 h, a neutral pH value and a temperature of 32 °C (OP V-B)), a stable yield of around 150 $mg_{tSCFAs} \bullet g_{VS}^{-1}$ (f_{DOC} = 84 %) at a 3-day SS_{ar} can be achieved. The gas yields of CH_4 and H_2 were less than 5 $NmL_{CH4} \bullet g_{VS}^{-1}$ and 1 $NmL_{H2} \bullet g_{VS}^{-1}$ (see Table 3).

When comparing modes with similar conditions, specifically OP II-D and III-B, certain discrepancies in yields were obvious. There appears to be an overall decrease in Y_{tSCFAs},Y_{SCFAs} and Y_{CH4} for III-B compared to II-D (see Fig. 6A, B and Table 3). In addition, overall gas production appears to have decreased compared to OP II. OLR was notably different and could not be maintained constant during the course of different OPs. It is known that, for biogas production in anaerobic digestion, significant increase in OLR leads to reactor failure due to SCFAs accumulation causing hyper acidification. A study conducted by Bouzas et al. on PS in a continuous system showed increasing yields of tSCFAs with increasing OLRs at HRT of 6 and 10 days. However, at 8 and 10 days, the yields were more or less stable (Bouzas et al., 2002). In this study, yields of tSCFAs were stable in the range of 12 to 22 $g_{VS} \bullet L^{-1} \bullet d^{-1}$ (results are not shown). OLR does not appear to have a major influence in this study at

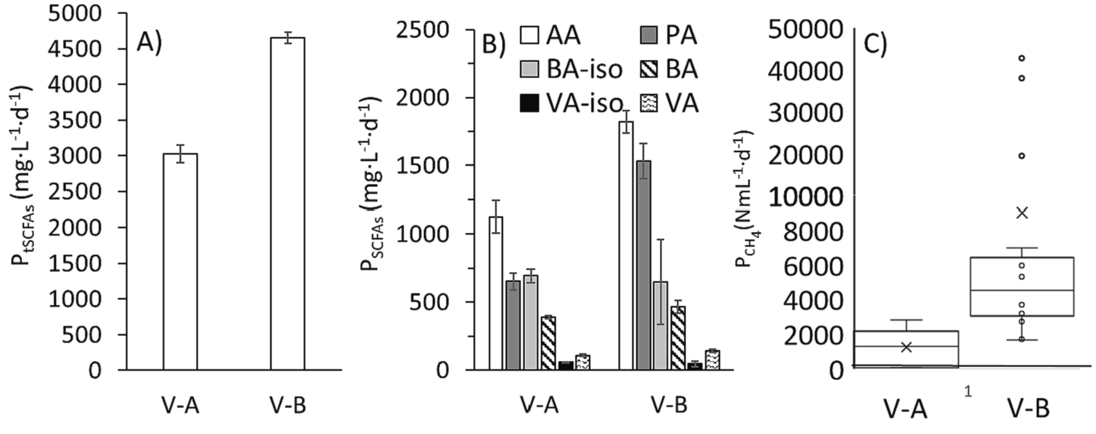

Fig. 5. . Daily productivities of A) total short-chained fatty acids (tSCFAs) (P_{tSCFAs}), B) individual SCFAs (P_{SCFAs}) C) CH_4 (P_{CH4}) for different temperatures (operation phase (OP) V-A; T = 25.2 °C and V-B; T = 32.4 °C) at hydraulic retention time of 36 h during 3-day arbitrary steady-state (refer Table 1 for detailed parameters).

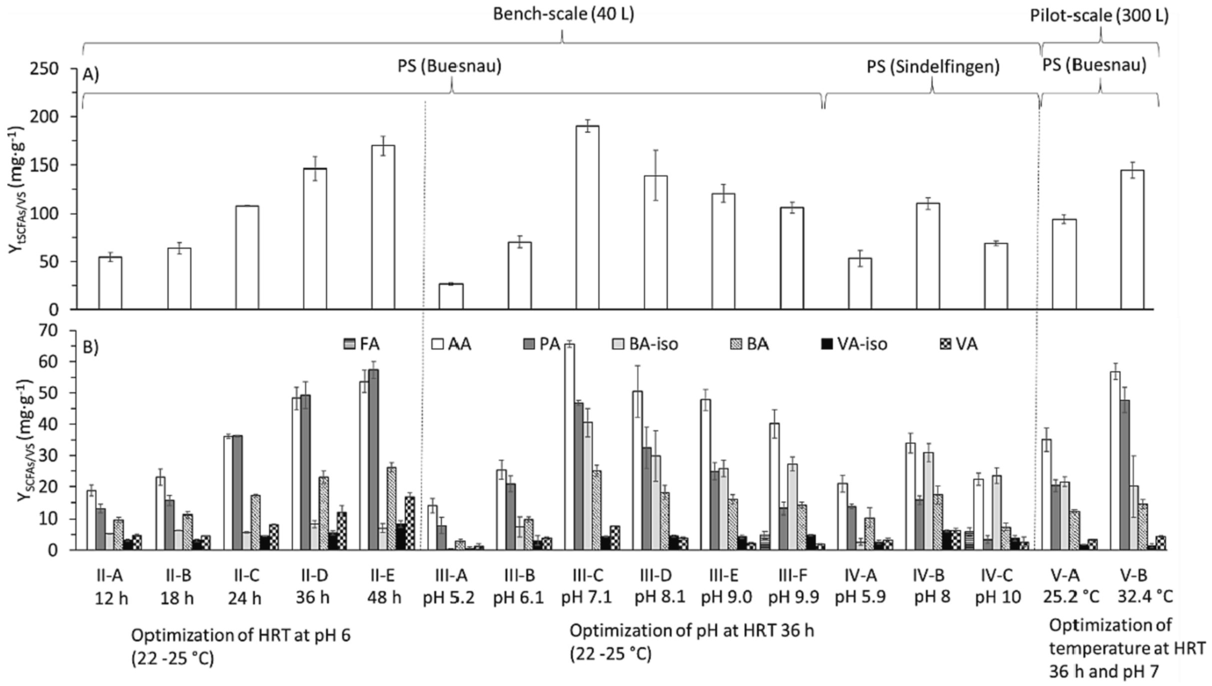

Fig. 6. . Yield of A) total short-chained fatty acids (tSCFAs) (Y_{tSCFAs}) and B) individual SCFAs (Y_{SCFAs}) for optimization of hydraulic retention time (HRT) at pH 6 (operation phase (OP)-II), optimization of pH at HRT of 36 h for two different primary sludge (OP-III and IV) and, optimization of temperature at HRT 36 h and pH 7 (OP-V) (refer Table 1 for detailed parameters).

Table 3
. Average yield of CH_4 and H_2 ($Y_{CH4,\ H2,\ avg/VS}$), yield of NH_4^+ ($Y_{NH4+/VS}$) for optimization of HRT at pH 6 (OP-II), optimization of pH at HRT 36 h for two different PS (OP-III and IV) and, optimization of temperature at HRT 36 h and pH 7 (OP-V) (refer Table 1 for detailed parameters). HRT: hydraulic retention time; OP: Operation phase.

OP	$Y_{NH4+/VS}$ (mg•g^{-1})	$Y_{CH4,\ avg/VS}$ (NmL•g^{-1})	$Y_{H2,\ avg/VS}$ (NmL•g^{-1})
II-A	4 ± 1	0.6	–
II-B	7 ± 1	0.8	–
II-C	13 ± 1	1.7	–
II-D	23 ± 3	3.2	–
II-E	20 ± 4	4.7	–
III-A	3 ± 0	0.4	–
III-B	9 ± 1	1.8	–
III-C	13 ± 2	2.1	–
III-D	8 ± 4	2.8	–
III-E	12 ± 1	0.4	0.3
III-F	10 ±	0.2	0.7
IV-A	9 ± 1	0.7	–
IV-B	16 ± 2	1.6	–
IV-C	8 ± 1	0.1	0.2
V-A	8 ± 1	0.3	–
V-B	7 ± 2	2.1	–

least within the tested range. Also, from the results of Wu et al., 2009 and Wu et al., 2010 done in a batch mode, wherein PS was obtained from the same location, the yields for tSCFAs and SCFAs remained constant for PS (at similar pH and temperature conditions) despite the significant difference in the concentration of initial VSS added (which deviated by 60 %).

Interestingly, yields of ammonium were notably higher at II-D compared to III-B (see Table 3). The higher $Y_{NH4+/VS}$ shows that proteins were degraded more in OP II-D compared to III-B. When comparing sludge from Buesnau (OPs II, III and V), C/N ratio was low in OP-II indicating the higher nitrogen content (in the form of proteins). C/N ratio is crucial in influencing metabolic pathways and producing different SCFAs (Rughoonundun et al., 2012). The authors noted that higher molecular weight-SCFAs such as VA and VA-iso were shown to decrease at higher C/N ratios due to a lack of proteinaceous substances. On the contrary, OP IV (PS from Sindelfingen) also had low C/N ratio but $Y_{NH4+/VS}$ was lower. The yields of SCFAs at OP IV (PS from Sindelfingen) were moderately lower but followed a similar trend as OP III. The composition and yields of SCFAs was also quite similar but there was a notable difference in yields of PA especially at the alkaline range (AA was also quite low). Such differences in SCFAs production between sludge types was also noted by Yuan et al., 2010.

The distribution of macromolecules (among proteins, carbohydrates and fats) in PS are critical in producing SCFAs and require further investigations, since higher fractions of fats (specifically long-chain fatty acids (LCFAs)) have been shown to negatively impact the process of acidification (Miron et al., 1999; Vidal et al., 2000). Higher yields of VA and VA-iso along with a higher yield of NH_4^+ could hint that the fraction of lipids (LCFAs) is lower in OP II compared to OPs III, IV, and V. However, it is necessary to investigate the composition of PS (distribution of carbohydrates, proteins and fats) and the changes it can undergo during various seasons as it can increase or decrease the yield. Da Ros et al., 2020 and Crutchik et al., 2018 showed that PS enriched with cellulose (cellullosic PS) is able to hydrolyze more efficiently into SCFAs (especially AA and PA). Indeed, the composition of PS has a major influence on the overall yields that can be achieved as there was notable difference in the distribution of SCFAs. Also, shifts in fermentation parameters like pH, temperature, and nutrient ratios can lead to the selective growth of different organisms, and, thus, cause a significant shift in biocoenosis that produces different metabolites (Chen & Chang, 2017; Huang et al., 2021b; Lin et al., 2018; Wang et al., 2021). Some of these studies could show certain commonalities in phyla that could dominate at defined conditions like pH and temperature in a complex substrate like sludge. Although biocoenosis composition has not been investigated in this study, the stability and ratio of SCFAs production achieved at different conditions with low CVs could potentially hint at the prevalence of specific phyla. In that case, significant changes in phyla may not occur during optimized pH and temperature conditions and could maintain a stable yield over long-term functioning of dark fermentation.

In any case, dark fermentation has to be adapted to the changing conditions of PS. The highest yield that was achieved at bench-scale was approximately 280 $mg_{tSCFAS,\ COD}\bullet g_{VS}^{-1}$ at 3-day SS_{ar} (pH 7.1, T = 25 °C, HRT = 36 h; OP III-C). AA and PA had the highest yields of 68 $mg_{tSCFAS,\ COD}\bullet g_{VS}^{-1}$ and 69 $mg_{tSCFAS,\ COD}\bullet g_{VS}^{-1}$, respectively. Yields achieved with PS at lab-scale at pH 10 was between 190 and 300 $mg_{tSCFAS,\ COD}\bullet g_{VS}^{-1}$ (Wang et al., 2021; Wu et al., 2009, 2010). At pilot-scale, at SS_{ar}, the yield was found to be around 207 $mg_{tSCFAS,\ COD}\bullet g_{VS}^{-1}$ (at pH 6.9, T = 32 °C, HRT = 36 h; OP V-B). The yields for AA and PA were 61 $mg_{COD}\bullet g_{VS}^{-1}$ and 72 $mg_{COD}\bullet g_{VS}^{-1}$. Similar conditions (pH 7, T = 35 °C) were also tested by Christensen et al., 2022 at HRT of 72 h at pilot-scale (3 m^3) and the yield was 80 % lower compared to this study.

Performance of the reactor was rather stable but it is unclear how extremely low sludge loading periods (high dilution) could impact the overall stability. Although alkaline pH-ranges may enhance yields at longer HRTs, there are practical disadvantages which can be addressed with the optimized conditions in this study such as, smaller reactor size (due to short HRTs) which provides an advantage in terms of operability and low chemical requirement (neutral pH conditions) to maintain a stable pH-value. Also, when run at stable alkaline pH conditions, a post-acidification step might be necessary if downstream commercial product recovery steps, like bioelectrochemical H_2-production, demand a near neutral pH-range (Liu et al., 2012). With the yields achieved in this study, the process itself is a practical option for H_2 production after subsequent membrane separation. For instance, using an MEC, Liu et al., 2012 were able to achieve a yield of 1.2 $mL_{H2}\bullet mg_{COD}^{-1}$ at tSCFAs concentration of 6042 $mg_{tSCFAS,\ COD}\bullet L^{-1}$ with AA (2544 $mg_{AA,\ COD}\bullet L^{-1}$) and PA (1143 $mg_{PA,\ COD}\bullet L^{-1}$) as the dominant SCFAs. At OP V-B, 1943 $mg_{AA,\ COD}\bullet L^{-1}$ and 2315 $mg_{PA,\ COD}\bullet L^{-1}$ could be achieved, making such a process very plausible. In both cases, the distribution among SCFAs were quite similar. In case of PHA, it is necessary to have a higher PA/AA ratio to enhance polymer production with enhanced physical and mechanical properties (Jiang & Chen, 2009). Frison et al., 2015 was able to achieve a consistent distribution among 3-hydroxybutyrate, 3-hydroxyvalerate and 3-hydroxyhexanoate (56 %:42 %:2 %) at PA/AA ratio of 1.1 $g_{COD,\ PA}\bullet g_{COD,\ AA}^{-1}$. In this study, PA/AA ratio of 1.2 $g_{COD,\ PA}\bullet g_{COD,\ AA}^{-1}$ (OP V-B) can be achieved at optimized conditions and could also be well suitable for PHA recovery. Also, SCFAs which are easily degradable can be used as carbon source for biological nutrients removal, specifically denitrification.

4. Conclusions

Dark fermentation can be a viable preliminary approach for valorizing organic carbon in the solids treatment step in a WWBr. The requirement of HRT less than 48 h, pH 7 and a temperature of 32 °C makes this process a feasible pre-treatment step from an economic standpoint. Maximum yields achieved at pilot-scale amounted to around 150 $mg_{SCFAs}\bullet g_{VS}^{-1}$ with yields of AA and PA reaching up to 57 and 48 $mg\bullet g_{VS}^{-1}$, respectively. The highest productivity of AA and PA at pilot-scale were 1.2 and 1 $g\bullet L_{reactor}^{-1}\bullet d_{HRT}^{-1}$ and could make commercial products recovery a potential option in the solids stream.

CRediT authorship contribution statement

Nikhil Shylaja Prakash: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Peter Maurer:** Funding acquisition, Conceptualization. **Harald Horn:** Writing – review & editing, Supervision, Funding acquisition. **Andrea Hille-Reichel:** Writing – review & editing, Validation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2024.130467.

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